Chapter 10
Biosynthesis of Nucleic Acids: Replication

SUMMARY

Section 10.1

- Before cells divide, they must synthesize a new copy of DNA. This process is called replication.
- When DNA is used as a template to synthesize RNA, the process is called transcription, and is the subject of the next chapter.
- The RNA sequence of messenger RNA is used to direct the synthesis of proteins in a process called translation.

Section 10.2

- When a molecule of DNA is replicated, each of the two strands is used as a template to create a complementary strand. When a cell divides into two, each of the two cells has one of the original template strands and one of the new strands. This process is called semiconservative replication.
- When DNA molecules are replicated, the strands are separated at origins of replications. Synthesis occurs in both directions from the origin along replication forks.

Section 10.3

- To achieve 5' → 3' synthesis of DNA on two strands that are antiparallel, DNA Polymerase synthesizes one strand continuously and the other discontinuously.
- The strand synthesized continuously is called the leading strand and the one synthesized discontinuously is called the lagging strand.
- The pieces of DNA formed discontinuously are called Okazaki fragments and they are later joined together by DNA Ligase.
- The reaction of DNA synthesis involves the nucleophilic attack of the 3'-hydroxyl of one nucleotide on the γ-phosphate of the incoming nucleoside triphosphate.
- There are at least 5 DNA polymerases in E. coli, called Pol I through Pol V. Pol III is the principal enzyme responsible for synthesis of new DNA, and it is a multiple subunit enzyme.
- DNA Polymerases I and II are involved in proofreading and repair processes.
- All DNA synthesis requires an RNA primer.

Section 10.4

- DNA replication is carried out by a multiprotein complex called the replisome. Besides the DNA polymerases themselves, many other proteins are involved in replication. DNA gyrase induces negative supercoils in the DNA to compensate for the positive supercoils that would form due to strand separation, and helicase induces strand separation. Single-stranded binding proteins protect the single stranded regions from nucleases.
- Primase primes the synthesis of the lagging strand by the formation of primers, and DNA ligase links pieces of newly formed DNA together.
- The primer and the proteins at the replication fork are called the primosome

Section 10.5

- Bases would be paired incorrectly during DNA synthesis about once for every 10^4 to 10^5 base pairs unless there were a mechanism to increase fidelity.
- Due to proofreading and repair, the number of incorrect bases is reduced to one in 10^9 to 10^{10}.
- Proofreading is the process where DNA Polymerase I removes incorrectly paired bases immediately after they are added to the growing chain.
- There are a variety of repair mechanisms that remove incorrect bases and nucleotides and replace them with the correct ones.

Section 10.6

- DNA recombination is a natural process in which genetic information is rearranged to form new associations.
- If the recombination involves a reaction between homologous sequences, then the process is called homologous recombination. When very different nucleotide sequences recombine, it is nonhomologous recombination**.**
- Recombination does not occur randomly around a chromosome. There are some areas of a chromosome, called hot spots, that are much more likely to show recombination.
- Recombination occurs by the breakage and reunion of DNA strands so that physical exchange of DNA parts takes place. The mechanism was deduced in 1964 by Robin Holliday and is referred to as the Holliday Model

Section 10.7

- Replication in eukaryotes follows the same general outline as replication in prokaryotes, with the most important difference being the presence of histone proteins complexed to eukaryotic DNA.
- Different proteins are used, and the system is more complex than it is in prokaryotes. Replication is controlled so that it occurs only once during a cell-division cycle, during the S phase.
- There are at least 19 different DNA polymerases in eukaryotes, of which five have been studied extensively -- α, β, γ, δ, and ε. Polymerase δ is the principal synthesizer of DNA and is the equivalent of Pol III in prokaryotes.

LECTURE NOTES

Most students will have seen much of the material in this chapter in earlier courses, particularly in beginning biology courses, but they are unlikely to have gone into any of the molecular details. The most difficult aspect of DNA replication for students to understand is frequently the distinct details of what is going on at the leading versus lagging strands. This is exacerbated by inherent difficulties in picturing things in three dimensions. Simple models using twisted strings are often helpful. The material on DNA replication may be expected to take between one and two lectures. The additional material on DNA repair will likely require most of a lecture period.

LECTURE OUTLINE

I. Flow of genetic information
    A. Replication
    B. Transcription
    C. Translation
II. DNA replication
    A. General considerations
        1. Separation of strands
        2. Directionality (5' —>3') of synthesis
        3. Guarding against errors
    B. Semiconservative replication
    C. Bidirectional replication
III. DNA polymerase
    A. Discontinuous synthesis of lagging strand
    B. DNA polymerases from E. coli
        1. Pol III core structure
        2. Necessity of a primer
        3. Proofreading functions
IV. Proteins required for replication
    A. Unwinding of the double helix
        1. DNA gyrase
        2. helicase
        3. SSB
    B. Primase reaction
    C. Synthesis and linking of new DNA strands
V. Proofreading and repair
    A. Mutations as errors in replication
    B. Nick translation
    C. Common mutagens
    D. Mismatch repair
    E. Methylation as a way to distinguish strands
    F. Base excision repair
    G. Nucleotide-excision repair
    H. Non-homologous DNA end-joining
VI. Recombination
    A. Homologous vs nonhomologous
    B. Location of crossing over
    C. Holliday model
    D. Proteins involved in recombination
VII. Eukaryotic DNA replication
    A. Replicons
    B. Cell cycle control
    C. Eukaryotic DNA polymerases
    D. The eukaryotic replication fork

ANSWERS TO PROBLEMS

10.1 The Flow of Genetic Information in the Cell

1. Replication is the production of new DNA from a DNA template. Transcription is the production of RNA from a DNA template. Translation is the synthesis of proteins directed by mRNA, which reflects the base sequence of DNA.
2. False. In retroviruses, the flow of information is RNA → DNA.
3. DNA represents the permanent copy of genetic information, whereas RNA is transient. The cell could survive production of some mutant proteins, but not DNA mutation.

10.2 Replication of DNA

4. The semiconservative replication of DNA means that a newly formed DNA molecule has one new strand and one strand from the original DNA. The experimental evidence for semiconservative replication comes from density-gradient centrifugation (Figure 10.3). If replication were a conservative process, the original DNA would have two heavy strands and all newly formed DNA would have light strands.
5. A replication fork is the site of formation of new DNA. The two strands of the original DNA separate, and a new strand is formed on each original strand.
6. An origin of replication consists of a bubble in the DNA. There are two places at opposite ends where new polynucleotide chains are formed (Figure 10.4).
7. Separating the two strands of DNA requires unwinding the helix.
8. If the original Meselson–Stahl experiment had used longer pieces of DNA, the results would not have been as clear-cut. Unless the bacteria were synchronized as to their stage of development, the DNA could have represented several generations at once.
9. Replication requires separating the strands of DNA. This cannot happen unless the DNA is unwound.

10.3 DNA Polymerase

10. Most DNA-polymerase enzymes also have exonuclease activity.
11. DNA polymerase I is primarily a repair enzyme. DNA polymerase III is mainly responsible for the synthesis of new DNA. See Table 10.1.
12. The processivity of a DNA polymerase is the number of nucleotides incorporated before the enzyme dissociates from the template. The higher this number, the more efficient the replication process.
13. The reactants are deoxyribonucleotide triphosphates. They provide not only the moiety to be inserted (the deoxyribonucleotide) but also the energy to drive the reaction (dNTP → inserted NMP + PP_i, PP_i → $2P_i$).
14. Hydrolysis of the pyrophosphate product prevents the reversal of the reaction by removing a product.
15. One strand of newly formed DNA uses the 3′-to-5′ strand as a template. The problem arises with the 5′-to-3′ strand. Nature deals with this issue by using short stretches of this strand for a number of chunks of newly formed DNA. They are then linked by DNA ligase (Figure 10.5).

16. The free 3′ end is needed as the site to which added nucleotides bond. A number of antiviral drugs remove the 3′ end in some way.
17. The large negative $\Delta G°$ ensures that the back reaction of depolymerization does not occur. Energy overkill is a common strategy when it is critically important that the process does not go in the reverse direction.
18. Nucleophilic substitution is a common reaction mechanism, and the hydroxyl group at the 3′ end of the growing DNA strand is an example of a frequently encountered nucleophile.
19. Some enzymes have a recognition site that is not the same as the active site. In the specific case of DNA polymerase III, the sliding clamp tethers the rest of the enzyme to the template. This ensures a high degree of processivity.

10.4 Proteins Required for DNA Replication

20. All four deoxyribonucleoside triphosphates, template DNA, DNA polymerase, all four ribonucleoside triphosphates, primase, helicase, single-strand binding protein, DNA gyrase, DNA ligase.
21. DNA is synthesized from the 5′ end to the 3′ end, and the new strand is antiparallel to the template strand. One of the strands is exposed from the 5′ end to the 3′ end as a result of unwinding. Small stretches of new DNA are synthesized, still in an antiparallel direction from the 5′ end to the 3′ end and are linked by DNA ligase. See Figure 10.5.
22. DNA gyrase introduces a swivel point in advance of the replication fork. Primase synthesizes the RNA primer. DNA ligase links small, newly formed strands to produce longer ones.
23. In the replication process, the single-stranded portions of DNA are complexed to specific proteins.
24. DNA ligase seals the nicks in newly formed DNA.
25. The primer in DNA replication is a short sequence of RNA to which the growing DNA chain is bonded.
26. Specific enzymes exist to cut the DNA and give a supercoiled configuration at the replication fork that allows replication to proceed.
27. Polymerase III does not insert a deoxyribonucleotide without checking to see that the previous base is correct. It needs a previous base to check even if that base is part of a ribonucleotide.
28. DNA polymerases have a very common structure that is often compared to a right hand, with domains referred to as the fingers, palm, and thumb. The active site where the polymerase reaction is catalyzed lies in the crevice within the palm domain. The fingers domain acts in deoxynucleotide recognition and binding, and the thumb is responsible for DNA binding.
29. Recently it was concluded that three Pol III enzymes are associated with the replisome instead of two.
30. Fluorescent labeling
31. A clamp loader is necessary because the sliding clamp of DNA Polymerase is a closed circle. It would not be able to get around the DNA without an enzyme to open it up.

10.5 Proofreading and Repair

32. When an incorrect nucleotide is introduced into a growing DNA chain as a result of mismatched base pairing, DNA polymerase acts as a 3′-exonuclease, removing the incorrect nucleotide. The same enzyme then incorporates the correct nucleotide.
33. In *E. coli,* two different kinds of exonuclease activity are possible for DNA polymerase I, which functions as a repair enzyme.
34. An exonuclease nicks the DNA near the site of the thymine dimers. Polymerase I acts as a nuclease and excises the incorrect nucleotides, then acts as a polymerase to incorporate the correct ones. DNA ligase seals the nick.
35. In DNA, cytosine spontaneously deaminates to uracil. The presence of the extra methyl group is a clear indication that a thymine really belongs in that position, not a cytosine that has been deaminated.
36. About 5000 books: 10^{10} characters/error × 1 book/(2×10^6 characters) = 5×10^3 books/error.
37. 1000 characters/second × 1 word/5 characters × 60 seconds/minute = 12,000 words/minute.
38. 1 second/1000 characters × 10^{10} characters/error × 10^7 seconds/error = 16.5 weeks/error nonstop.
39. Prokaryotes methylate their DNA soon after replication. This aids the process of mismatch repair. The enzymes that carry out the process can recognize the correct strand by its methyl groups. The newly formed strand, which contains the incorrect base, does not have methyl groups.
40. DNA is constantly being damaged by environmental factors and by spontaneous mutations. If these mistakes accumulate, deleterious amino acid changes or deletions can arise. As a result, essential proteins, including those that control cell division and programmed cell death, are inactive or overactive, eventually leading to cancer.
41. Prokaryotes have a last-resort mechanism for dealing with drastic DNA damage. This mechanism, called the SOS response, includes the crossing over of DNA. Replication becomes highly error-prone, but it serves the need of the cell to survive.
42. Non-homologous DNA End Joining (NHEJ) or recombination.
43. Ku70/80, DNA ligase IV, and several others.
44. It is error-prone as the repair proceeds without a template
45. It binds the broken ends of the DNA so replication can continue

10.6 Recombination

46. Recombination that involves a reaction between homologous sequences.
47. They used two different phages to infect bacteria. One of the phages had light DNA and one had heavy DNA. Without recombination, the light DNA would always package into light viral particles, and the heavy DNA would package into heavy viral particles. This would lead to only two populations of phages after infection. Their results showed, however, that there were intermediate combinations that had DNA of different weights. This demonstrated that the phage DNA was recombining.

48. Similar to the experiment described in 47 above, using heavy isotopes demonstrated the semi-conservative nature of replication. Intermediate weight products of replication demonstrated that progeny DNA contains one parental strand and one new strand.
49. Recombination occurs by the breakage and reunion of DNA strands so that physical exchange of DNA parts takes place. The mechanism was deduced in 1964 by Robin Holliday and is referred to as the Holliday Model.

10.7 Eukaryotic DNA Replication

50. Eukaryotes usually have several origins of replication, whereas prokaryotes have only one.
51. The general features of DNA replication are similar in prokaryotes and eukaryotes. The main differences are that eukaryotic DNA polymerases do not have exonuclease activity. After synthesis, eukaryotic DNA is complexed with proteins; prokaryotic DNA is not.
52. Histones are proteins complexed to eukaryotic DNA. Their synthesis must take place at the same rate as DNA synthesis. The proteins and DNA must then assemble in proper fashion.
53.

(a) Eukaryotic DNA replication must deal with histones; the linear DNA molecule in eukaryotes is a much larger molecule and requires special treatment at the ends.
(b) Special polymerases are used in the organelles.

54. Eukaryotes have more DNA polymerases, which tend to be larger molecules. Eukaryotic DNA polymerases tend not to have exonuclease activity. There are more origins of replication in eukaryotes and shorter Okazaki fragments. See Table 10.5.
55. Mechanisms exist to ensure that DNA synthesis takes place only once in the eukaryotic cell cycle, during the S phase. Preparation for DNA synthesis can and does take place in the G1 phase, but the timing of actual synthesis is strictly controlled.
56. If the telomerase enzyme were inactivated, DNA synthesis would eventually stop. This enzyme maintains the 3′ template end strand so that it does not undergo degradation with each round of DNA synthesis. The degradation in turn arises from the removal of the RNA primer with each round of DNA synthesis.
57. If histone synthesis took place faster than DNA synthesis, it would be highly disadvantageous to invest the energy required for protein synthesis. The histones would have no DNA with which to bind.
58. Replication licensing factors (RLFs) are proteins that bind to eukaryotic DNA. They get their name from the fact that replication cannot proceed until they are bound. Some of the RLF proteins have been found to be cytosolic. They have access to the chromosome only when the nuclear membrane dissolves during mitosis. Until they are bound, replication cannot occur. This property links eukaryotic DNA replication and the cell cycle. Once RLFs have bound, the DNA is then competent for replication.
59. It is faster in prokaryotes. The DNA is smaller, and the lack of compartmentalization within the cell facilitates the process. DNA replication in

eukaryotes is linked to the cell cycle, and prokaryotic cells proliferate more quickly than those of eukaryotes.

60. In reverse transcriptase action, the single RNA strand serves as a template for the synthesis of a single DNA strand. The DNA strand, in turn, serves as the template for synthesis of the second strand of DNA.
61. Circular DNA does not have ends. This removes the necessity for maintaining the 3′ template end on removal of the RNA primer. Telomeres and telomerase are not needed with circular DNA.
62. The presence of a DNA polymerase that operates only in mitochondria is consistent with the view that these organelles are derived from bacteria incorporated by endosymbiosis. The bacteria were originally free-living organisms earlier in evolutionary history.
63. The hypothesis that RNA was the original molecule of heredity, and was the first molecule that took simple compounds and turned them into larger molecules with a function.
64. Because finding that RNA can self-replicate leads credence to the RNA world hypothesis and brings us a step closer to understanding how evolution began.